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Nucleotide Pools and Adenylate Energy Charge in Balanced and Unbalanced Growth of *Chromatium*

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Adenine nucleotide pools and their energy charge were measured during balanced and unbalanced growth of photoheterotrophic *Chromatium* cultures. The methods used involved rapid sampling, accurate to within 1 s, from isotopically labeled cultures followed by chromatographic separation of individual nucleotides. During balanced growth, both energy charge and adenosine triphosphate (ATP) concentrations, whether expressed as a function of cell protein or intracellular water, were slightly higher in limiting light intensities than in cultures growing at their maximal rate in bright light. The ATP found corresponded to 4.67 ± 0.08 nmol/mg of protein or 1.34 ± 0.57 mM for low-light cells and to 4.41 ± 0.58 mmol/mg of protein or 0.85 ± 0.12 mM for high-light cells. Corresponding energy charges were 0.85 ± 0.02 and 0.81 ± 0.02. Illumination shifts caused differential synthesis of photosynthetic pigments lasting 2 to 3 h without corresponding perturbation of adenine nucleotide levels. Cultures in intermittent illumination were severely affected by some cycle durations; they had abnormal morphology and very high bacteriochlorophyll-to-protein ratios. In such cultures, energy charge and nucleotide concentrations were within normal limits and relaxed to the dark steady state during the dark periods. Arsenate at AsO_4^{3-} to PO_4^{3-} ratios of 10:1 in the medium retarded growth, but no abnormality of charge or quantity of phosphate-containing nucleotides was found. These experiments therefore suggest that, within experimental error, neither the size nor the charge of the adenylate pools governs growth rate in *Chromatium*. Moreover, these parameters do not appear to be concerned in regulating the synthesis of photosynthetic apparatus in this organism.

Adenine nucleotides play a central role in the collection and redistribution of metabolic energy, and therefore are especially well suited for playing a role in the regulation of enzyme activities and consequently of growth rate. A number of investigations have placed emphasis on the concentration of adenosine triphosphate (6) or on the ratio of adenine nucleotides (adenosine mono-, di-, and triphosphates [AMP, ADP, and ATP]), particularly as expressed in the formulation (ATP + 0.5 ADP)/(ATP + ADP + AMP), or the energy charge of the adenylate pool (1, 2) as being of primary importance in such regulation.

There have been some differences in experimental findings, but the greater number of investigations have emphasized the similarities of both pool sizes and nucleotide distribution in bacterial cultures growing under a variety of conditions (summarized in 2), although larger differences in the pools of other metabolites may be found (10). Substantial changes in adenine nucleotide distribution are found only if the cells are subjected to a severe shift in experimental conditions, leading to at least a temporary cessation of growth (2, 12).

Relative constancy in adenine nucleotide pools and distribution has been demonstrated also in photosynthetic bacteria, in which distinct systems are concerned with ATP formation and ATP consumption (16, 20). Such organisms growing photosynthetically respond to differences in incident light intensity not only by variation in growth rate but also by a change in photosynthetic apparatus content, being more strongly pigmented in dim

light than in bright light (3). Several groups of workers have postulated a function of nucleotide concentration or distribution in regulating photosynthetic apparatus synthesis (18–21, 24, 25).

We have previously reported on nucleotide pools in *Chromatium* cultures in balanced growth at different rates controlled by the intensity of light falling on the culture (16); these experiments did not support such hypotheses.

In this paper, further results are presented for cultures undergoing metabolic stresses which might be expected to alter the distribution of nucleotides. Since experiments previously reported (9, 16) have emphasized that the concentrations of adenine nucleotides are redistributed within seconds of darkening either growing or nongrowing suspensions, it is essential to employ a reproducible sampling method in measuring steady-state concentrations of nucleotides. The procedure used in these studies minimizes changes of illumination during sampling and gives rapid quenching of culture samples. The results emphasize again that adenine nucleotide levels, whether expressed as concentrations or ratios, are closely controlled, and do not support a direct role of these parameters in regulation either of growth rates or photosynthetic apparatus synthesis.

MATERIALS AND METHODS

Growth of cultures. *Chromatium* strain D was grown photoheterotrophically with succinate, pyruvate, or equal concentrations of these compounds as carbon sources, as previously described (16). Cultures were grown anaerobically in completely filled 18-ml screw-capped tubes maintained at 30 C and illuminated with 40-cm 30-W tungsten filament lumiline lamps placed 10 cm in front of culture tubes. This resulted in a light intensity of 2.4 mW/cm^2 at the tube surface. Lower incident light intensities were obtained by wrapping the tubes in one or two fine metal-mesh screens, painted black. Light intensities were calibrated by use of a YSI Radiometer with a Wratten 88A filter and a 5-cm water filter. Intermittent illumination was obtained by switching the lamps on and off at the desired times by use of a recycling timer (Singer Industrial Timer Corp., Parsippany, N.J.).

Growth rates were estimated from turbidity measurements of the culture tubes in a Fisher Electrophotometer II with a 650-nm filter. All nucleotide measurements were made on cultures in balanced growth under the conditions specified. Three to four serial 1:10 transfers were necessary to achieve this. The protein content of all cultures used in nucleotide determinations was measured directly, since this gave more reliable values for cell density than turbidity measurements in culture tubes.

Water space of Chromatium. Intracellular unbound water of suspensions was calculated from the difference between the volume of ^{14}C-ethylene glycol and of ^{14}C-inulin passing through a silicone oil barrier with the cells from a suspension of *Chromatium*. Ethylene glycol, since it mixes with the cell water but is not concentrated, measures both intra- and extracellular water, whereas the inulin, which does not penetrate and is not metabolized, gives a value for extracellular water passing the barrier. Cultures were concentrated by centrifugation to approximately 10 mg of protein/ml and were incubated with ethylene glycol-*1,2-*^{14}*C* (0.1 μCi/ml; 5.4 mM) or COOH-^{14}C-inulin (0.1 μCi/ml; 4 μm) for 20 min at room temperature. Quadruplicate portions of 0.1 ml of these suspensions were then layered on the liquid surface in 0.4-ml polyethylene microfuge tubes (A. H. Thomas) containing 0.1 ml of 8% (wt/vol) sucrose, 2% Triton X-100, and a silicone oil barrier as described by Gaensslen and McCarty (7). After centrifugation for 15 s at full speed in a Coleman microfuge, the tubes were frozen. Top and bottom aqueous layers were separated by cutting the tube and were counted by use of Bray's fluid in a Packard liquid scintillation counter.

Nucleotide determinations. Preliminary experiments showed that incorporation of $^{32}PO_4^{3-}$ into 5% trichloroacetic acid-precipitable material increased at the same rate as optical density only after 2.5 to 3 doublings in radioactive medium, indicating only slow equilibration between isotope and internal pools of phosphorylated compounds. Cultures were therefore grown in the labeling medium for at least three doublings, and sampling for nucleotide extraction was started when the cultures contained no more than 60 to 70 μg of cell protein per ml. The concentration of phosphate used (0.1 mM) permitted exponential growth to about 200 μg of protein per ml.

The experimental procedure was in part described previously (16). At the start of the experiment, culture from the growth tube was drawn into a 5-ml gas-tight repeater syringe with a 5-cm, 20-gauge fixed needle delivering 0.1-ml portions (Hamilton Co., Whittier, Calif.) which had been flushed 10 times with prepurified nitrogen. Illumination was maintained at the same level as during growth while the syringe was filled and for a subsequent 20-min equilibration period before any samples were taken. During this period, and during any time longer than 30 s between samples, the syringe needle was protected from air by being put into a small test tube through which nitrogen was passing. After any period of several minutes between samples, one 0.1-ml sample was discarded to eliminate cells which had been lodged in the needle for a prolonged time. Control experiments showed that growth continued at the same rate in test tubes and in the syringe for a period of at least 15 h.

For extraction of nucleotides, samples of culture were injected into 2.5 μliters of 16 N formic acid in polyethylene microfuge tubes and left to stand for 30 min at room temperature before being centrifuged. Although ejection of the sample and mixing with acid required no more than 0.1 s, the precision of sample timing in dark periods was not better than 1 s. Cell-free medium was obtained by sampling into

empty microfuge tubes and centrifuging within 30 s; there were, however, no indications of rapid changes in quantity or kind of extracellular labeled materials in any of these experiments. Samples of 10 μliters of the acid extracts and culture supernatant fluids, containing of the order of 0.1 pmol of individual nucleotides, were chromatographed on polyethyleneimine cellulose-coated plastic sheets (20 by 20 cm; Brinkmann Instruments, Inc., Westbury, N.Y.) with the use of the LiCl and formic acid solvents of Randerath and Randerath (18). Radioactive areas were located by radioautography, cut out, and counted in a liquid scintillation counter. In some experiments in which adenine nucleotides only were required, standards and radioactive samples were co-chromatographed and were located by ultraviolet absorption. The specific activity of the $^{32}PO_4^{3-}$ in the medium was calculated from chemical determinations of inorganic phosphate at the time of making and from radioactive counting under the same conditions and at the same time as experimental samples. The specific activity was about 150×10^9 counts per min per μmol in all experiments.

Chemical determinations. Bacteriochlorophyll was estimated in acetone-methanol extracts according to Cohen-Bazire et al. (3), with the use of an extinction coefficient of 75 mM^{-1} cm^{-1}. Protein in acetone-methanol-extracted pellets was measured by the method of Lowry et al. (15). Inorganic phosphate was determined colorimetrically by the procedure of Dryer et al. (5). Inorganic arsenate was measured by the same procedure with 4 h instead of 10 min allowed for color development.

Radioactive materials. $H_3{}^{32}PO_4$, carrier-free, ethylene glycol-*1,2-*^{14}C, and COOH-^{14}C-inulin were obtained from International Chemical and Nuclear, Inc., Irvine, Calif.

RESULTS

Adenine nucleotide concentrations. We reported previously (16) that the adenylate energy charge is the same in cultures growing at a rate of 0.2 doubling/h in high light intensity and at a rate of 0.1 doubling/h in limiting light, even though the low-light culture had three times the specific bacteriochlorophyll content of the high-light culture. Microscopic examinations of slowly growing cultures of *Chromatium* showed that the cells were considerably smaller than those in rapidly growing cultures, as is the general rule with other types of bacteria. This, coupled with the higher content of photosynthetic apparatus, suggested that the intracellular water content, as a function of cell protein, might differ substantially in the two types of culture, and that the concentration of adenine nucleotide might, even with constant energy charge, yet be significantly different. Measurements of intracellular water for cells with doubling rates of 0.2 and 0.1/h gave values of 5.17 ± 0.04 and 3.49 ± 1 μliters/mg of protein, respectively. Table 1 summarizes the results of 11 (6 + 5) experiments in which adenylates were measured in cultures in which growth rate was controlled by light intensity. Measurements of the absolute quantities of nucleotides are subject to considerably greater error than energy charge, since recoveries must be quantitative and specific activity and protein determinations must be exact. Energy charge, on the other hand, is independent of specific activity and cell concentration and can be computed with less than quantitative recoveries if it is assumed that the same proportion of each nucleotide is lost from any one sample. In practice, duplicate chromatograms from any one cell extract and duplicate samples from steady-state cultures both gave values for quantities of nucleotides which usually agreed within 10%. The major variations therefore appear to come from specific activity and protein measurements in independent experiments rather than from chromatography and recovery in any one series. The data in Table 1 show that low light-grown cells have a slightly higher content of ATP, expressed either on the basis of cell protein or of concentrations, and that they also have a significantly higher energy charge. The values for total nucleotides differ somewhat from those reported earlier, which were based on fewer measurements. The improvements in experimental technique in obtaining the results given here are reflected in the much smaller variances.

Energy shifts. Light intensities of 2.4 and 0.4 mW/cm^2 permit the same growth rate in balanced cultures of *Chromatium* (16), although the specific bacteriochlorophyll content is doubled at the lower light intensity. Further reduction in light intensity effects a further increase in pigment content, but it also affects growth rate. When a balanced high-light culture was screened so as to reduce the light intensity, net protein synthesis was reduced by about 90% for a period of 2.5 to 3 h, during which preferential synthesis of pigments occurred. Subsequently, balanced growth resumed at the rate and with the specific pigment content expected in the new light intensity (B. J. Reilinger, personal communication; Fig. 1). A shift-down in energy supply therefore redirects macromolecular synthesis, and it has been postulated that either the concentration of ATP (19, 21) or the adenylate energy charge (26; H. Gest et al., Proc. 2nd Int. Congr. Photosynthesis Res., Stresa, Italy, 1971, in press) may act as the signal for this change. When adenine nucleotide concentrations and energy charge were measured during energy shift of a culture held in the sampling syringe, a small but significant drop in ATP and in

TABLE 1. *Adenine nucleotides of Chromatium grown in saturating or limiting light intensities*

Light intensity during growth (mW/cm²)	Energy charge	Adenine nucleotides (nmol/mg of protein ± SD)[a]		
		ATP	ADP	AMP
2.4	0.81 ± 0.02	4.41 ± 0.58 (0.85 ± 0.12)	1.17 ± 0.58 (0.22 ± 0.11)	0.59 ± 0.04 (0.11 ± 0.01)
0.2	0.85 ± 0.02	4.67 ± 0.08 (1.34 ± 0.57)	1.01 ± 0.08 (0.29 ± 0.15)	0.36 ± 0.04 (0.11 ± 0.06)

[a] The equivalent values expressed as the millimolar concentration are given in parentheses.

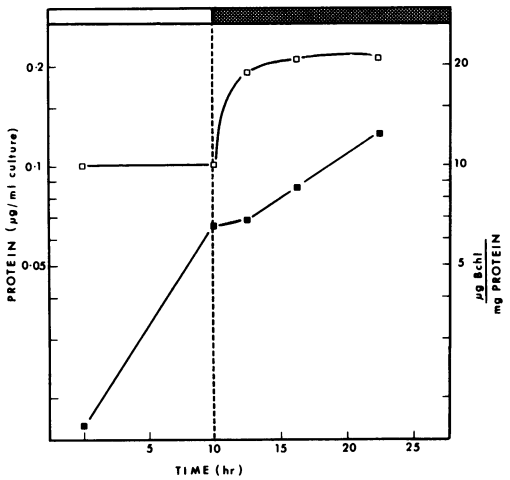

FIG. 1. *Protein and bacteriochlorophyll (Bchl) synthesis during illumination shift-down. A culture was grown in balanced growth in high light (2.4 mW/cm², indicated by open bar at top of figure) and transferred to the syringe as described in the text. After about two doublings, the syringe barrel was screened to reduce the light intensity to 0.2 mW/cm², indicated by stippled bar. Samples of 1 ml were withdrawn for determinations of bacteriochlorophyll and protein: (■) protein; (□) specific bacteriochlorophyll content.*

energy charge was observed immediately after reduction of light intensity. Within 40 s, both had returned to their initial values, and they remained at the values characteristic of growing cells, or slightly above, during the remainder of the unbalanced period, during which preferential synthesis of bacteriochlorophyll was occurring, and during resumption of protein synthesis (Fig. 1 and 2).

During a complementary experiment (not shown), in which a culture growing at the lower light intensity was shifted to more intense illumination, energy charge was also constant, without significant perturbation even in the first few seconds. This indicated that overproduction of ATP did not occur under such conditions, although bacteriochlorophyll synthesis was preferentially inhibited.

Table 2 also shows that values for ATP utilization in cultures which were shifted from low to high illumination were similar to those for down-shifts, but were greatly reduced in cultures whose growth rate was affected by intermittent illumination (see below).

An estimate of the rate of ATP utilization can be obtained at any time during such an experiment from the initial rate of decay after darkening of the culture. Such rates were estimated a few minutes before and at 30-min intervals after high to low light shifts in two experiments (Fig. 2, Table 2). Although sample timing was not precise enough to distinguish small differences in the rate of ATP consumption during the 3 h following the shift, it is clear that the cells were highly active metabolically even though little net protein synthesis was occurring during this period.

Growth in intermittent light. Other metabolic stresses affecting growth rate and specific pigment content have been applied to cultures in which nucleotide measurements have been made. As with *Rhodopseudomonas capsulata* (24), growth of *Chromatium* in intermittent illumination was severely affected at certain light-dark cycles, as is shown in Table 3. Such intermittently illuminated cultures were characterized also by high bacteriochlorophyll-to-protein ratios. Whereas a short-period cycle of 10 s light, 10 s dark gave growth rates close to one-half that of continually illuminated cells, in the most extreme case (90 s light, 90 s dark), balanced growth was never reached, and optical density increase stopped altogether after about six doublings. Cell morphology was grossly distorted (Fig. 3), and some cell lysis was apparent from the fact that supernatant fluids obtained by centrifuging such cultures were detectably colored and had absorption spectra characteristic of chromatophore preparations. Adenine nucleotide levels were followed during several cycles of a culture growing in a regime of 60 s light, 60 s dark (Fig. 4). When the light went on, ATP concentration and energy charge rose rapidly within 2 s and were maintained at normal values for growing cells until the end of the light period. Decay to the dark steady state was slow relative to that

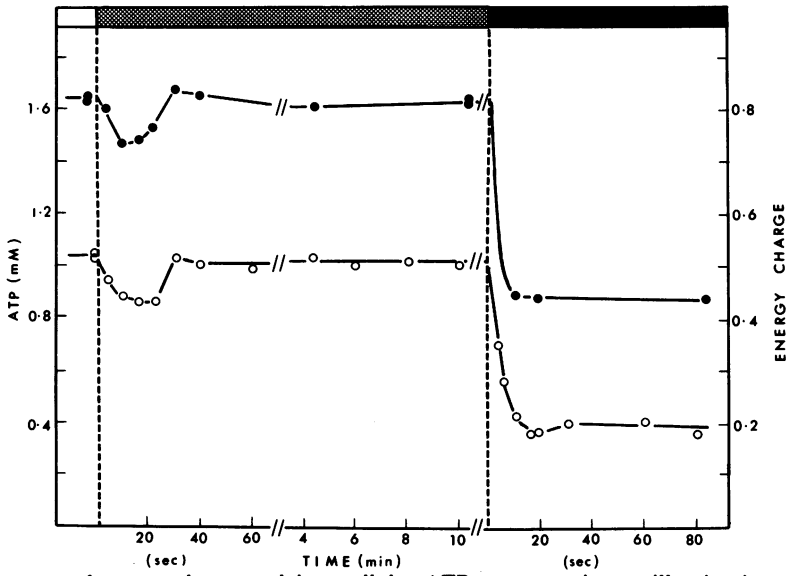

FIG. 2. *Response of energy charge and intracellular ATP concentration to illumination shift-down. A culture was grown in $^{32}PO_4^{3-}$-containing medium in high light (2.4 mW/cm², open bar at top of figure) and transferred to the syringe as described in the text. After 20 min of equilibration, the steady-state samples were taken. At the left dashed line, screens were placed over the culture to reduce the light intensity to 0.2 mW/cm² (stippled bar). Further samples were taken at the intervals shown. At the right dashed line, the light was turned out (black bar), and sampling was continued.* (●) *Energy charge;* (○) *ATP.*

TABLE 2. *Rate of ATP utilization in Chromatium cultures*

Culture conditions	Doublings/h	Initial rate of ATP decay[a]
Shift-down (2.4 → 0.2 mW/cm²)		
Zero time	0.154	0.5
60 min postshift		1.0
120 min postshift	0.054	0.56
180 min postshift		0.82
Shift-up (0.2 → 2.4 mW/cm²)		
Zero time		1.0
30 min postshift	0.095	0.97
Alternating light (2.4 mW/cm²)		
105 s on-15 s off	0.095	1.0[b]
60 s on-60 s off	0.014	0.21[c]

[a] Nanomoles per milligram of protein × seconds in the first 2 s after darkening.
[b] Average of decay rates in two light-dark cycles.
[c] Average of decay rates in three light-dark cycles.

TABLE 3. *Growth rates of Chromatium in intermittent light*[a]

Cycle length (s)	Doublings/h	Actual doublings/h[b] / Expected doublings/h
10 on, 10 off	.1	1.0
20 on, 20 off	.083	0.83
30 on, 30 off	.059	0.59
60 on, 60 off	.021	0.21[c]
90 on, 90 off	.015	0.15[c]

[a] Light intensity: 2.4 mW/cm².
[b] Expected doublings per hour was calculated by assuming maximal growth rate during light-on periods and zero growth in darkness.
[c] Balanced growth not achieved.

of more rapidly growing cultures (Table 2). Abnormal charge or nucleotide concentrations during the illuminated period therefore do not provide an explanation for the morphological changes or abnormally low growth rate of such cultures as compared with those subjected to light-dark cycles of shorter duration.

It remained possible, however, that the drop to low ATP level and energy charge which occurred during the dark period was itself causing severe effects on growth. This was investigated by use of a culture growing in a 105 s light, 15 s dark cycle, whose morphology and specific bacteriochlorophyll content were normal, and whose growth rate was about that expected if it was growing at a normal rate during the light periods. As shown in Fig. 5, both ATP and energy charge were normal during light periods and relaxed completely to a normal dark steady state during the 15-s dark period. Thus, a period of a few seconds of low ATP or energy charge did not have far-reaching

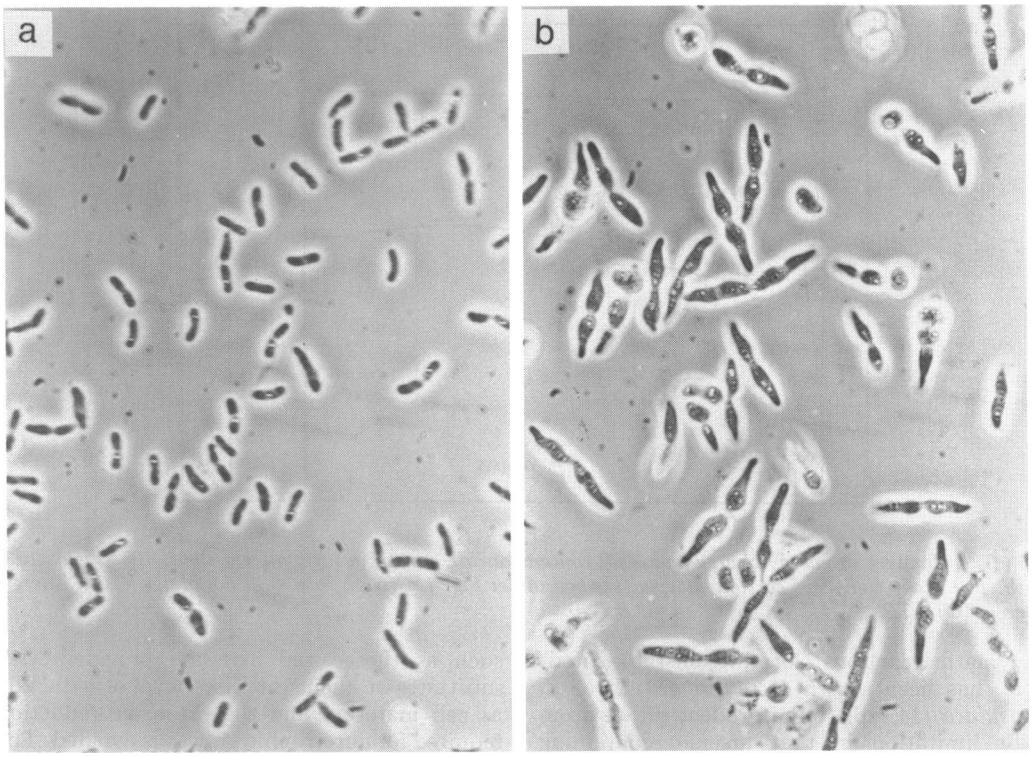

FIG. 3. *Photomicrographs of Chromatium cultures growing in continuous (a) and intermittent (b) light of 60 s light-60 s dark, as seen under 1,000 × phase contrast. Light intensity, 2.4 mW/cm².*

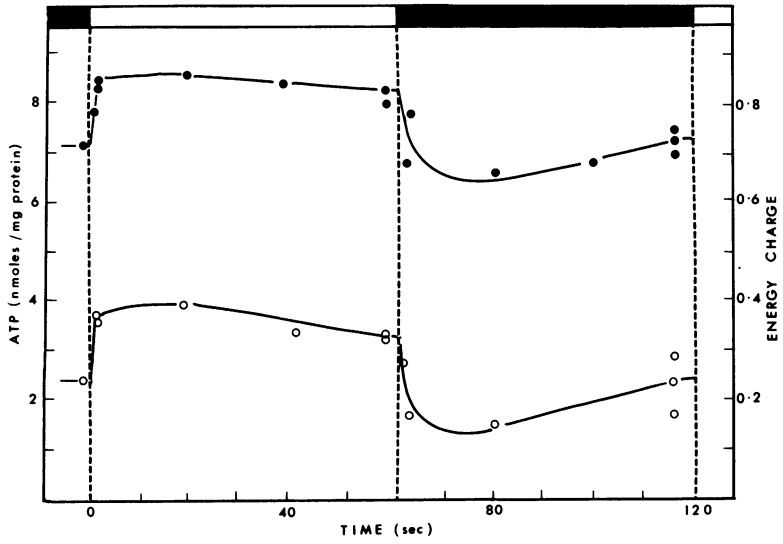

FIG. 4. *Changes of energy charge and intracellular ATP during growth in intermittent light: 60 s light, 60 s dark. The culture was labeled with $^{32}PO_4^{3-}$ for five doublings. Growth rate at time of experiment, approximately 0.002 doublings/h. The experiment was carried out as described for Fig. 2 and in the text. Dark bar at the top of the figure indicates dark period; open bar, light period. (●) Energy charge; (○) ATP. Light intensity, 2.4 mW/cm².*

damaging effects on growth rate or cell composition provided that it was not too long and was followed by an adequate illuminated recovery time.

Effect of arsenate. Arsenate, which is known to replace phosphate in a number of

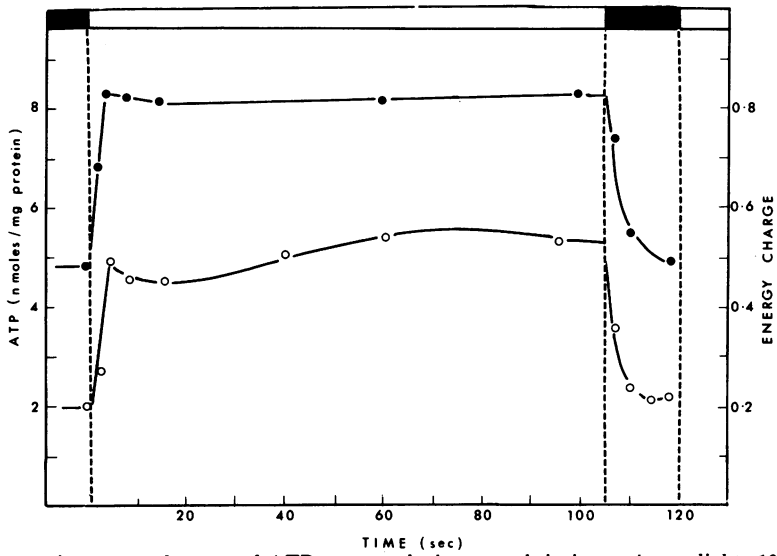

FIG. 5. *Changes in energy charge and ATP content during growth in intermittent light: 105 s light, 15 s dark. The experiment was carried out as described under Fig. 4. Growth rate, 0.17 doublings/h.* (●) *Energy charge;* (○) *ATP.*

enzymatic reactions, forming unstable products, has been shown to retard growth of *R. capsulata* (14, 26), but its effect differs from light limitation in that arsenate-containing cultures have a lower rather than a higher than normal specific bacteriochlorophyll content. This phenomenon was less marked with *Chromatium*. The concentration of arsenate required to affect growth was dependent on phosphate, and, in several experiments in the normal medium concentration of 0.1 mM PO_4^{3-}, 1 mM AsO_4^{3-} reduced the growth rate from 0.18 to 0.077 doubling or less per h. Measurements of intracellular arsenate and phosphate pools released from cells with 0.4 N formic acid indicated that the internal molar ratio was approximately 2, which agrees with the molar ratio in which effects on cell-free phosphorylating systems are observed (4).

Nucleotide levels in arsenate-inhibited cultures were measured both during continuous illumination and after a brief period of darkening. As shown in Table 4, energy charge and pool sizes of phosphate-containing nucleotides were substantially the same in the arsenate-retarded and control cultures. Changes in these parameters therefore do not account for the altered growth rate.

DISCUSSION

Photosynthetic bacteria have some definite advantages for the study of the regulatory role of nucleotides in growing cultures. In the first place, anaerobic photoheterotrophic cultures such as those used here are provided with substrates at about the same level of reduction as cell material, and the extensive reduction required for autotrophic growth is avoided. The nucleotide pools are thus used primarily for the macromolecular syntheses which add up to growth. Secondly, ATP is generated entirely through light-dependent reactions, and the illumination, and hence the energy supply, of the culture is readily controlled without mechanical disturbance. It is also possible to obtain a direct measurement of the turnover of the pools by measuring the initial rates of relaxation after darkening of the culture, and it is significant that triphosphate decay measured in this way correlates well with estimates of biosynthetic demands (16). Estimates of the turnover of nucleotide pools in *Escherichia coli* (12) have of necessity been indirect, involving oxygen uptake measurements and assumptions about oxidative phosphorylation efficiencies. The methods employed in this study also have some advantages over those used in other studies on photosynthetic bacteria (10, 12, 19–21). The isotopic labeling and chromatographic separation procedure make it possible to measure many nucleotides and phosphorylated derivatives in addition to adenine compounds in small samples and with a sensitivity at least as great as the luciferin-luciferase assay. Minimal disturbance of exponentially growing cultures is involved, and quenching of the sample is rapid. However, the simple manual sampling method we have used lacks

precision in timing, and it is probably for this reason that there is considerable variation in Table 2 in the initial rates of ATP decay on darkening similar cultures. A further problem lies in that approximately 10% of the volume ejected from the syringe in each sample comes from culture which had been resident in the unilluminated bore of the needle since the last sample was ejected. Therefore, the steady-state light values for nucleotide distribution and adenylate energy charge are all in need of some correction. It would appear that the energy charges given in Table 1, for instance, would be approximately 0.86 and 0.9 in fully illuminated high-light and low-light cultures, respectively, when corrected for this error.

The steady-state values for nucleotide concentrations and adenylate energy charge of balanced *Chromatium* cultures are similar to those reported previously (16) and to those in *E. coli* (2), and these results agree with the conclusion of the authors of the latter paper that the energy charge of the adenylate pool is between 0.8 and 0.9 in actively growing cultures. On darkening, a rapid fall in charge to a minimal value of 0.5 was observed, which could be maintained for at least 15 min provided that the cultures were kept anaerobic. A slow upward drift was observed in the dark if oxygen was present in such experiments, corresponding to the low dark aerobic phosphorylation rate observed with nongrowing cultures (8). Changes with similar time courses were observed in the other nucleotides resolved in the chromatographic system, involving substantial decreases in triphosphates and increases in di- and monophosphates. The stability of nucleotides in the dark steady state agrees with the results of Schmidt and Kamen (19) using *Chromatium* and also corresponds to the findings with *E. coli* at the onset of glucose starvation (2, 12). A further drop in charge occurred only after several hours in the investigation of Chapman et al. (2) and was associated with loss of viability.

A number of workers (2, 17) have reported on the presence of some metabolic intermediates in the external medium during growth. Phosphoenol pyruvate and phosphoglyceric acid were most prominent among extracellular compounds seen in this investigation. At least one-third of total quantities of these compounds, amounting to about 4 and 6 nmol/mg of protein, was external to the cells. Extracellular quantities remained constant, but intracellular concentrations increased slowly over a period of several minutes in darkness. Among nucleotides, extracellular triphosphates were barely detectable, but 25 to 35% of the total light steady-state diphosphates, and up to 50% of the monophosphates, were regularly found, regardless of the growth conditions. The extracellular appearance of these compounds therefore seems to be the result of selective export rather than of generalized leakiness, but its significance has not yet been investigated further.

TABLE 4. *Energy charge and nucleotide concentration in normal and arsenate-retarded cultures*[a]

Determination	Control[b]	Plus 1 mM arsenate[b]
Light steady-state energy charge	0.86	0.856
Nucleotides (nmoles/mg of protein)		
ATP	5.43	4.1
ADP	1.12	0.99
AMP	0.47	0.19[c]
Dark steady-state energy charge	ND[d]	0.53
Nucleotides (nmoles/mg of protein)		
ATP	—	1.35
ADP	—	1.55
AMP	—	0.91

[a] Light intensity: 2.4 mW/cm^2.

[b] Bacteriochlorophyll/protein in control = 20 μg/ml; in arsenate culture = 19.4 μg/ml.

[c] An equal quantity was also found extracellularly in this experiment.

[d] Not determined.

The interaction between nucleotides and enzymes usually involves magnesium ions. It was therefore important to establish the intracellular quantity of magnesium in the cultures, and investigations of total intracellular Mg^{2+} by atomic absorption spectrometry are in progress. In preliminary results, total intracellular Mg^{2+} concentrations of about 20 mM were found, and Mg^{2+} was tightly retained under the experimental conditions used here. Although part of the total is presumably in bound form, the total amount is substantially greater than the total phosphorus in the cell. It is therefore probable that there is sufficient free magnesium to facilitate interaction of the entire nucleotide pools with cell enzymes.

A major objective in this study was the measurement of nucleotides under a variety of conditions in order to elucidate any function of concentrations or energy charge in governing the relative photosynthetic apparatus content of cultures. It has been suggested that in other photosynthetic bacteria (14, 24, 26; Gest et al., in press) a lower energy charge or ATP content (19, 20) could be metabolic signals establishing the balance between photosynthetic membrane

and other cell component synthesis, with a lowered energy charge favoring the synthesis of the former over the latter. Our measurements, however, do not show this; rather, both energy charge and ATP content were somewhat higher in dimly lit than in brightly lit balanced cultures, although the differences observed are on the borderline of significance. Moreover, cultures shifted from bright to dim light, and undergoing a major redirection of biosynthesis lasting about 3 h, showed small nucleotide changes lasting less than 1 min. Subsequently, values were maintained within experimental error for at least 5 h, extending through the period in which preferential synthesis of photosynthetic pigments occurred, and into that of resumption of protein synthesis at the new rate. The significance of a slow rise in energy charge from 0.86 to 0.91 over a period of 3 h seen in some long shift-down experiments is not certain. It was not seen in another experiment in which occasional dark periods of 3 min were not imposed. Shift-up experiments, in which protein synthesis was maintained but bacteriochlorophyll synthesis was repressed until its specific content was reduced to that of high-light cultures, likewise showed no changes in nucleotide quantities. These findings are inconsistent with a function of energy charge or nucleotide concentration in regulating the synthesis of the photosynthetic membrane in *Chromatium* unless an extraordinarily sensitive site is involved. Our more recent energy charge measurements are reproducible to within 0.02. Although a function of a change not measurable within this error in our methods cannot be ruled out, the model systems examined by Atkinson and his group (13, 22) in general require a difference in energy charge of 0.1 or more to cause at most a doubling or halving in the rate of an enzyme reaction. One *Chromatium* enzyme, ribulose 5-phosphate kinase, has been shown to have a rather steeper energy charge response curve (11), but this enzyme is not critically involved in the incorporation of cell carbon in cultures grown under the conditions used in this study.

A number of other special conditions have been employed in attempts to disturb nucleotide balances. Intermittent illumination of certain cycles lowered the growth rate disproportionately and caused an increase in specific bacteriochlorophyll content, whereas arsenate poisoning usually caused a slight decrease in the latter as well as in growth rate, showing that specific pigment content was not obligatorily linked to growth rate. No abnormality in nucleotide quantity or balance was found in either of these types of culture, however, again indicating no direct involvement of these parameters in regulating growth rate or specific pigment content. One further possibility for perturbing nucleotide balances, which was suggested by experiments of Zilinsky et al. (26) showing that extracellular ATP inhibited growth and specific bacteriochlorophyll synthesis in *R. capsulata*, proved unusable in our hands with *Chromatium*. Although labeled adenine nucleotides were incorporated into nucleic acids during growth (Miović and Gibson, unpublished data), showing that they could enter the cell, 5 mM ATP did not affect growth or cell composition when added as Mg ATP.

In 1957, Cohen-Bazire, Sistrom, and Stanier (3) suggested that the redox state of a component of the electron-transport chain involved in both photosynthetic and oxidative ATP generation was responsible for suppressing or promoting the synthesis of photosynthetic membranes in *Rhodospirillum rubrum*. This model has received support from further investigations with *R. molischianum* (23) and a mutant of *R. spheroides* (25). Our results are not inconsistent with this model. They do, however, suggest a regulation point between this electron carrier and phosphate esterification, since only transient or no disturbance of energy charge was seen in shift experiments which cause a profound change in the balance of macromolecular synthesis. Both ATP production and utilization are clearly maintained at close to previous rates during such shifts, indicating that energy charge and ATP level are themselves closely regulated to within our experimental limitations. It appears that changes in these parameters are resisted and rapidly compensated for under all conditions investigated here except the extreme one of darkening, which must halt ATP synthesis entirely. Only under these circumstances are the full effects of energy charge on biosynthetic enzyme activity brought into play, resulting in stabilization of charge at a value of 0.5, rather than complete utilization of all immediately available nucleotide energy. It remains to be investigated whether the differences in our findings from those obtained by other workers are due to species variation or to the use of more precise experimental methods.

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LITERATURE CITED

1. Atkinson, D. E. 1968. The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. Biochemistry 7:4030-4034.

2. Chapman, A. G., L. Fall, and D. E. Atkinson. 1971. Adenylate energy charge in *Escherichia coli* during growth and starvation. J. Bacteriol. **108**:1072-1086.
3. Cohen-Bazire, G., W. R. Sistrom, and R. Y. Stanier. 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cell. Comp. Physiol. **49**:25-68.
4. Crane, R. K., and F. Lipmann. 1953. The effect of arsenate on aerobic phosphorylation. J. Biol. Chem. **201**:235-243.
5. Dryer, R. L., A. R. Tammes, and J. T. Routh. 1957. The determination of phosphorus and phosphatase with N-phenyl-p-phenylene-diamine. J. Biol. Chem. **225**:177-183.
6. Forrest, W. W. 1965. Adenosine triphosphate pools during the growth cycle in *Streptococcus faecalis*. J. Bacteriol. **90**:1013-1016.
7. Gaensslen, R. E., and R. E. McCarty. 1971. Amine uptake by chloroplasts. Arch. Biochem. Biophys. **147**:55-65.
8. Gibson, J. 1967. Aerobic metabolism of *Chromatium* sp. strain D. Arch. Mikrobiol. **59**:104-112.
9. Gibson, J., and S. Morita. 1967. Changes in adenine nucleotides of intact *Chromatium* produced by illumination. J. Bacteriol. **93**:1544-1550.
10. Harrison, D. E. F., and R. K. Maitra. 1969. Control of respiration and metabolism in growing *Klebsiella aerogenes*. The role of adenine nucleotides. Biochem. J. **112**:647-656.
11. Hart, B. A., and J. Gibson. 1971. Ribulose-5-phosphate kinase from *Chromatium* sp. strain D. Arch. Biochem. Biophys. **144**:308-321.
12. Holms, W. H., I. D. Hamilton, and A. G. Robertson. 1972. The rate of turnover of the ATP pool in *Escherichia coli* growing aerobically in simple defined media. Arch. Mikrobiol. **83**:95-109.
13. Klungsøyr, L., J. G. Hageman, L. Fall, and D. E. Atkinson. 1968. Interaction between energy charge and product feedback in the regulation of biosynthetic enzymes. Aspartokinase, phosphoribosyl adenosine triphosphate synthetase, and phosphoribosyl pyrophosphate synthetase. Biochemistry **7**:4035-4040.
14. Lien, S., A. San Pietro, and H. Gest. 1971. Mutational and physiological enhancement of photosynthetic energy conversion in *Rhodopseudomonas capsulata*. Proc. Nat. Acad. Sci. U.S.A. **68**:1912-1915.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
16. Miović, M. L., and J. Gibson. 1971. Nucleotide pools in growing *Chromatium* strain D. J. Bacteriol. **108**:954-956.
17. Moses, V., and P. B. Sharp. 1972. Intermediary metabolite levels in *Escherichia coli*. J. Gen. Microbiol. **71**:181-190.
18. Randerath, E., and K. Randerath. 1964. Resolution of complex nucleotide mixtures by two-dimensional anion-exchange thin-layer chromatography. J. Chromatogr. **15**:126-129.
19. Schmidt, G. L., and M. D. Kamen. 1971. Control of chlorophyll synthesis in *Chromatium vinosum*. Arch. Mikrobiol. **76**:51-64.
20. Schön, G. 1969. Der Einfluss der Kulturbedingungen auf den ATP-ADP-uAMP-Spiegel bei *Rhodospirillum rubrum*. Arch. Mikrobiol. **66**:348-364.
21. Schön, G., and R. Bachofen. 1970. Der Einfluss der Sauerstoffpartialdrucks und der lichtintensität auf der ATP-spiegel in Zellen von Athiorhodaceae. Arch. Mikrobiol. **73**:34-46.
22. Shen, L. C., L. Fall, G. M. Walton, and D. E. Atkinson. 1968. Interaction between energy charge and metabolite modulations in the regulation of enzymes of amphibolic sequences. Phosphofructokinase and pyruvate dehydrogenase. Biochemistry **7**:4041-4045.
23. Sistrom, W. R. 1965. Effect of oxygen on growth and the synthesis of bacteriochlorophyll in *Rhodospirillum molischianum*. J. Bacteriol. **89**:403-408.
24. Sojka, G. A., and H. Gest. 1968. Integration of energy conversion and biosynthesis in the photosynthetic bacterium *Rhodopseudomonas capsulata*. Proc. Nat. Acad. Sci. U.S.A. **61**:1486-1493.
25. Wittenberg, T., and W. R. Sistrom. 1971. Mutant of *Rhodopseudomonas spheroides* unable to grow aerobically. J. Bacteriol. **106**:732-738.
26. Zilinsky, J. W., G. A. Sojka, and H. Gest. 1971. Energy charge regulation in photosynthetic bacteria. Biochem. Biophys. Res. Commun. **42**:955-961.